EFFECT OF SELECTED PROBIOTIC SUPPLEMENTATION ON PSYCHO-PHYSIOLOGICAL VARIABLES OF UNIVERSITY ATHLETES

A SYNOPSIS

Submitted in partial fulfillment of the requirements for the degree of

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CERTIFICATE

I hereby declare that the synopsis **"**EFFECT OF SELECTED PROBIOTIC SUPPLEMENTATION ON PSYCHO-PHYSIOLOGICAL VARIABLES OF UNIVERSITY ATHLETES**"** being submitted in the partial fulfilment of the requirement for the degree of Doctor of Philosophy in Physical Education under the Department of Exercise Physiology, Lakshmibai National Institute of Physical Education Gwalior - 474002 during the academic year 2022-2023.

It is a bona fide record of original work carried out under the supervision of Dr. Birendra Jhajharia (LNIPE, Gwalior, M.P.) and has not been submitted in full or part to any other university or institute for a degree or diploma.

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ABSTRACT

The gut microbiota—a diverse ecosystem of microorganisms residing in the human gastrointestinal tract—plays a vital role in regulating physiological, metabolic, and psychological health. Recent research has highlighted its influence on athletic performance, immune modulation, mental well-being, and metabolic balance. Probiotics, defined as live beneficial microorganisms, have emerged as a promising strategy to modulate gut microbiota and support psycho-physiological outcomes. In athletes, probiotics have been linked to reduced gastrointestinal distress, enhanced immune response, improved mood, better sleep quality, and favorable lipid profile changes. Despite these promising findings, human studies investigating the isolated and combined effects of exercise and probiotic supplementation on gut microbiota and related health parameters remain limited. The current study aims to evaluate the effect of selected probiotic supplementation on anxiety, stress, mood, sleep quality, and lipid profile parameters in university-level male athletes aged 18–27. Using a randomized controlled design, the study will assess the impact of probiotic strains from the Lactobacillus and Bifidobacterium genera. This research will provide insights into the potential of probiotics as a non-pharmacological intervention to enhance psychological and physiological health in athletes, with implications for sports nutrition, training recovery, and overall well-being.

Keywords:

Gut microbiota, probiotics, anxiety, stress, mood, sleep quality, lipid profile, athletes, Lactobacillus, Bifidobacterium, sports nutrition, psycho-physiological health.

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CHAPTER I

INTRODUCTION

Studies have recognized that gut microbiota can influence both human health and athletic performance. In the 2010s, advancements in high-throughput sequencing technologies and bioinformatics, along with the development of methods to quantify microbial metabolites, enabled large-scale population studies on the human microbiota. These studies have significantly enhanced our understanding of the microbiota's role in human physiology and its dysregulation in various chronic diseases (MetaHIT Consortium et al., 2010). The gut microbiota, a complex and dynamic community of microorganisms including bacteria, fungi, viruses, and archaea, colonizes the intestinal surface and comprises approximately 10^{14} microbes. In healthy individuals, the dominant phyla include Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes (Wosinska et al., 2019). These microorganisms are responsible for numerous essential functions, such as the synthesis of vitamins and amino acids, the regulation of immune responses, oxidative stress, and the maturation of the nervous system through neuroactive molecules. Additionally, they contribute to digestion, absorption, and nutrient fermentation, playing a critical role in maintaining energy balance and homeostasis (Clark & Mach, 2016).

In 2018, the International Olympic Committee (IOC) acknowledged probiotics as a supplement with moderate effectiveness in supporting immune health, particularly in reducing respiratory tract infections. However, it also emphasized the need for further research on their effects on gut-related issues and infections. A position stand by the International Society of Sports Nutrition (ISSN) further elaborated on the potential of probiotic supplementation to promote health, enhance athletic performance, and aid recovery (Miles, 2020). This article investigates the link between gut microbiota, chronic diseases, and athletic performance. Mechanistic studies using gnotobiotic models have provided insights into molecular interactions and opened avenues for gut microbiota modulation. It reviews current knowledge on how gut microbiota influences chronic conditions and athletic outcomes and examines the synergistic effects of physical activity and nutritional strategies in modulating gut microbiota for improved health and performance. The underlying hypothesis is that these interventions, when combined, may exert complementary and enhanced effects. This evolving knowledge base holds promise for developing targeted interventions involving dietary modifications, supplementation, and exercise regimens to prevent or manage chronic diseases and boost athletic performance.

Modulation of Gut Microbiota by Exercise and Nutrition for Health and Performance

Emerging evidence suggests that gut microbiota diversity and functionality can be influenced by diet and exercise, affecting overall physiological outcomes. This makes the gut microbiota a promising target for interventions involving nutrition and physical activity. However, while these interventions are often implemented separately, few human studies have examined their combined effects, aside from some studies involving probiotics in high-level athletes (Jäger et al., 2019). Donati Zeppa et al. (2019) indicated the potential of nutritional supplements in modifying the gut microbiota to enhance athletic performance, yet no human study has compared the isolated and combined effects of structured physical training and nutritional strategies on gut microbiota. Animal studies, such as those by Plissonneau et al. (2021), have demonstrated possible additive and synergistic outcomes from such combined interventions.

Probiotics, Athletes, and Performance

Jäger and colleagues noted the prevalence of gastrointestinal (GI) issues among endurance athletes, including nausea, vomiting, and diarrhoea, often exacerbated by overtraining, stress, sleep deprivation, and environmental conditions. These factors may lead to immunosuppression and increase vulnerability to infections. Consequently, probiotics are increasingly used in sports to address GI symptoms and reduce the incidence of respiratory infections (Jäger et al., 2019). Although outcomes depend on the strain, dosage, duration, and form of administration, studies suggest that prolonged use of multi-strain probiotics, especially in sachets or fermented foods, yields better results (Leite et al., 2019). The positive effects of probiotics are attributed to enhanced gut barrier function, immune modulation, increased production of short-chain fatty acids (SCFAs), reduced gut permeability, and increased mucosal immunity. Lamprecht et al. proposed that probiotics might activate Toll-like receptor 2 (TLR2), promoting tight junction protein expression and reducing endotoxemia and GI symptoms.

West et al. reported a seven-fold increase in Lactobacillus among athletes following 11 weeks of *Lactobacillus fermentum* supplementation. Martarelli et al. also observed increased fecal Lactobacillus counts after supplementation during intense physical activity. Though these changes did not always correlate with performance improvements, some evidence indicates that probiotics may indirectly aid performance. For example, Huang et al. found that six weeks of *Lactobacillus plantarum* TWK10 supplementation increased time to exhaustion and reduced serum lactate. Another study using *L. plantarum* PS128 demonstrated reduced markers of muscle damage and oxidative stress post-race. These findings suggest that probiotics might support recovery and muscle repair, potentially via enhanced protein synthesis (Gepner et al.,

2017; Wosinska et al., 2019).

Optimising Health Through Gut Microbiota Modulation via Supplements and Exercise

Lifestyle interventions such as diet and exercise have shown beneficial effects on metabolic health in conditions like obesity, type 2 diabetes, and inflammatory bowel diseases. The American College of Sports Medicine recommends 150 minutes per week of low- to moderate-intensity training for individuals with metabolic disorders. High-intensity interval training (HIIT) is also gaining attention for its efficiency in reducing fat mass, improving glucose metabolism, and modulating gut microbiota (Demangel et al., 2017).

Gut Microbiota and Mental Health in Adults

Gut microbiota may also influence mental health through neural, endocrine, and immune pathways (Järbrink-Sehgal & Andreasson, 2020). Disruption of the intestinal barrier can lead to systemic cytokine release, which communicates with the brain via the vagus nerve or across the blood-brain barrier. Additionally, gut-derived metabolites may reach the brain through the bloodstream, while brain activity, in turn, affects gut microbiota via hormonal and behavioral feedback. This bidirectional relationship highlights the potential for microbiota-targeted interventions to support mental well-being alongside physical health.

STATEMENT OF THE PROBLEM

The statement of the problem is to study the Effect of selected probiotic supplementation on psycho-physiological variables of university athletes.

OBJECTIVES OF THE STUDY

The objectives of the study are:

1. To find out the effect of probiotic supplementation on the Anxiety of university athletes.
2. To find out the effect of probiotic supplementation on the stress levels of university athletes.
3. To find out the effect of probiotic supplementation on the mood of university athletes.
4. To find out the effect of probiotic supplementation on the sleep quality of university athletes.
5. To find out the effect of probiotic supplementation on selected lipid profile

parameters of university athletes.

DELIMITATIONS

Since the study will be conceptualized with a very specific purpose for selected subjects. The research scholar confined this study to the following areas:

1. The study will be confined to male Athletes.
2. The study will be delimited to players who have participated in inter-university or state-level championships.
3. The study will be delimited to the age range between 18-27 years.
4. The study is limited to probiotic supplementation containing strains of Lactobacillus or Bifidobacterium class.
5. The study will further be delimited to anxiety, stress, mood, and sleep quality.
6. The study will further be delimited to assessing Total Cholesterol, HDL, LDL, VLDL Triglyceride in lipid profile parameters.

LIMITATIONS

1. Questionnaire has its limitations; any bias that might have entered the subject on this account may be considered a limitation to this study.
2. Life lifestyle of the athletes is beyond the control of the researcher. Training more than the stated duration, Socio-economic and religious factors, which cannot be controlled by the scholar, might affect the responses of the subjects; these are considered as the limitations for this study.
3. Certain factors like geographic conditions, illness, diet taken other than the general one on weekends, even after being instructed by the researcher not to consume, would affect the results of the study, so it will be considered a limitation of the study.
4. The use of antibiotics by experimental group subjects, even after being instructed by the researcher not to use them, as it affects the result of probiotic supplementation, will be considered a limitation of the study.
5. The subjects consume commercially available probiotic supplements such as probiotic yogurt, Yakult, even after being instructed by the researcher not to use them

on their own, will be considered a limitation of the study.

HYPOTHESIS

Based on the review of related literature, books' depiction, scientific facts available, expert opinion, and the researcher's understanding of this research study following hypotheses are formulated:

1) There will be a significant effect of probiotic supplementation on Anxiety.
2) There will be a significant effect of probiotic supplementation on stress levels.
3) There will be a significant effect of probiotic supplementation on mood.
4) There will be a significant effect of probiotics supplementation on sleep quality.
5) There will be a significant effect of probiotic supplementation on selected Lipid profile parameters.

DEFINITION AND EXPLANATION OF IMPORTANT TERMS

PROBIOTICS

Probiotics are defined as "live microorganisms which when consumed in adequate amounts confer a health benefit on the host" (Hill et al. 2014).

ANXIETY

Bouras and Holt (2010) explain Anxiety, "Anxiety is a feeling of uneasiness and worry, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing".

STATE ANXIETY

"State anxiety is the actual feeling of tension and nervousness. It is a transitory state" (Martens, 1973).

TRAIT ANXIETY

"A tendency to perceive competitive situations with a feeling of apprehension or tension"

(Martens, 1973).

STRESS

Lazarus (1966) defined stress as a state of imbalance within an organism that is elicited by an actual/perceived disparity between environmental demands and the organism's capacity to cope with these demands, and is manifested through a variety of physiological, emotional, and behavioural responses. The most explicit definition is given by Lazarus."

MOOD

Mood is generally defined as a state of emotional or affective arousal of varying, impairment duration (Weinberg and Gould, 2007)

SLEEP QUALITY

Sleep quality refers to how well the sleeper reports having slept after the sleep episode or to sleep in general. (Geddes & Andreasen, 2020)

SUPPLIMENTATION

The word "supplementation" means adding to something. Food supplements are a concentrated source of essential nutrients in the form of capsules, medicines, or injections. (IAPSM's Textbook of Community Medicine, n.d,2019)

LIPID PROFILE

A lipid profile is a group of tests that measure various blood fats. It is one indicator of a person's risk for cardiac and vascular disease. (Timby & Smith, 2013)

TOTAL CHOLESTEROL

Total cholesterol, the total amount of cholesterol in your blood based on your HDL, LDL, and triglycerides numbers. (Centers for Disease Control and Prevention, USA)

LOW DENSITY LIPOPROTEIN

Low-density lipoprotein is a primary carrier of cholesterol that can bind to receptors in many tissues, including the liver, muscle, and arterial walls (Dufaux, Assmann, and Hollman 1982)

VERY LOW-DENSITY LIPOPROTEIN

Very low-density lipoprotein VLDL are triglyceride-rich -rich lipoprotein, which comprises

10% to 15% of total serum cholesterol (Mary,2009)

HIGH DENSITY LIPOPROTEIN

High-density lipoprotein (HDL) or “good” cholesterol. HDL is known as “good” cholesterol because high levels of it can lower your risk of heart disease and stroke. (Centers for Disease Control and Prevention, USA)

TRIGLYCERIDE

Triglycerides, a type of fat in your blood that your body uses for energy. The combination of high levels of triglycerides with low HDL cholesterol or high LDL cholesterol levels can increase your risk for a heart attack and stroke. (Centers for Disease Control and Prevention, USA)

SIGNIFICANCE OF THE STUDY

1. The study will provide knowledge about probiotics on health.
2. The study will provide information about available probiotics supplements in the market.
3. The study will further motivate the athletes to use easily available probiotics.
4. The study will help coaches and physical educationists to use probiotics to enhance athletic performance by improving gut health.

CHAPTER-II

Review of Literature

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have gained increasing attention for their role in physical and psychological well-being. They are commonly found in fermented foods, cultured milk products, and are widely incorporated into infant nutrition. **Shi et al. (2016)** highlight that probiotic, often referred to as "health-friendly bacteria," offer a broad spectrum of health-promoting properties, including the prevention of gastrointestinal disorders, enhancement of immune function, alleviation of lactose intolerance, and maintenance of intestinal microbial balance. Furthermore, probiotics demonstrate antihypercholesterolemic and antihypertensive effects, mitigate postmenopausal symptoms, and reduce travel-related diarrhoea. Emerging evidence also suggests their potential in treating dermatological and oral health conditions. Notably, the gut-brain axis modulation by probiotics has been proposed as a novel therapeutic strategy for managing anxiety and depression.

Adikari et al. (2020) conducted a randomized, double-blind, placebo-controlled trial to examine the impact of probiotic supplementation on anxiety-related physiological parameters in competitive football players. Over eight weeks, 20 male athletes consumed either *Lactobacillus casei* Shirota (3×10^{10} CFU) or a placebo. While heart rate and electrodermal responses showed no statistically significant changes, improvements in cognitive function, particularly in reaction time during the digit vigilance test, were observed in the probiotic group. Notably, significant modulation was seen in brain wave activity, especially in theta and delta waves, at week four, suggesting potential psychophysiological benefits of probiotics in athletic performance.

Golunuk et al. (2017) explored the effects of fermented beverages—kefir and boza—on biochemical markers in individuals undergoing aerobic training. In a study involving 36 sedentary male participants aged 18–25, significant improvements were noted in the kefir group for ALT and total oxidant status (TOS), while the boza group demonstrated significant changes in triglyceride, VLDL, chloride, and creatinine levels. The findings indicate that natural probiotic beverages can positively influence metabolic health in physically active individuals, supporting their use over synthetic performance enhancers.

Salleh et al. (2021) assessed the effects of *Lactobacillus casei* Shirota supplementation on psychological parameters and physical fitness in university badminton players. The six-week intervention showed a significant reduction in competitive anxiety (16%) and perceived stress (20%) among the probiotic group. Moreover, there was a notable improvement in aerobic capacity (5.9%), though no significant effects were observed on speed, strength, leg power, or agility. These results support the potential of probiotics in enhancing psychological resilience and cardiorespiratory fitness in athletes.

Salehzadeh (2015) evaluated the influence of probiotic yogurt on lipid profiles, C-reactive protein (CRP), and athletic performance. In a controlled study of normal-weight aerobic athletes, probiotic yogurt consumption led to significant increases in HDL and reductions in CRP levels. Importantly, the reduction in CRP was associated with improved physical performance, with the probiotic group showing a 28-second improvement in endurance records, as compared to a 2-second gain in the control group.

Kekkonen et al. (2007) investigated the effect of *Lactobacillus rhamnosus* GG on gastrointestinal (GI) symptoms and upper respiratory tract infections (URTIs) in marathon runners. Although no significant differences were observed in the prevalence of GI symptoms or URTIs, the probiotic group experienced a reduction in the duration of GI episodes by 33% during training and 57% during the follow-up period. These results suggest a potential role of probiotics in minimizing the severity of gastrointestinal distress among endurance athletes.

Järbrink-Sehgal and Andreasson (2020) conducted two randomized controlled trials exploring the influence of probiotics on brain function in healthy adults. Participants who consumed *Bifidobacterium longum* 1714TM exhibited altered neural oscillations in the frontal and cingulate cortices during rest and post-stress conditions. These changes were associated with enhanced vitality and reduced mental fatigue. In a parallel trial, individuals receiving a multispecies probiotic showed improved mood and distinctive patterns in brain responses to emotional tasks, suggesting the potential cognitive-emotional benefits of probiotics.

Lew et al. (2019) carried out a 12-week, double-blind, placebo-controlled trial on stressed adults to evaluate the strain-specific effects of *Lactobacillus plantarum* P8. The study demonstrated significant reductions in perceived stress, anxiety, and total psychological distress, as measured by the DASS-42. Furthermore, probiotic supplementation led to significant decreases in pro-inflammatory cytokines (IFN-γ and TNF-α), along with

enhancements in memory and cognitive performance, particularly in verbal learning and social cognition, with gender-specific effects observed.

Mohammadi et al. (2016) explored the impact of probiotic yogurt and a multispecies probiotic capsule on mental health and hypothalamic–pituitary–adrenal (HPA) axis activity in petrochemical workers. After six weeks, both probiotic groups showed significant improvements in general mental health (GHQ scores) and reduced levels of depression, anxiety, and stress (DASS scores). The were no significant improvements observed in the control group. These findings underscore the beneficial psychophysiological effects of probiotic interventions in occupational settings.

CHAPTER III

METHODOLOGY

This chapter describes the methodology and procedure of the study. It includes the selection of subjects, variables, the collection of data, and statistical techniques to be employed for the analysis of data.

SELECTION OF SUBJECTS

For the study, male university athletes will be selected purposively. Subjects will then be randomly divided into two groups: Experimental and Control group subjects should have a similar routine, similar diet, and training for at least 2 hours.

SELECTION OF VARIABLES

From the researcher's understanding of the problem and based on discussion with experts, gleaning through the literature following variables have been chosen:

A. INDEPENDENT VARIABLES

a) Probiotic supplementation

B. DEPENDENT VARIABLES

Psychological variable

a) Anxiety
b) Stress
c) Mood
d) Sleep quality

Physiological variable

a) Total cholesterol
b) Triglycerides
c) High density lipoprotein
d) Low-density lipoprotein
e) Very low-density lipoprotein

CRITERION MEASURE

ANXIETY

Anxiety will be assessed through the Sports Anxiety, (SAS-2) Sports Anxiety Scale-2developed by Ronald E. Smith, Frank L. Smoll, Sean P. Cumming, and Joel R. Gross bard with 15 statements on a 4-point Likert scale anchored by Not at all, A Little Bit,

Pretty Much, Very Much was used.

STRESS

Stress level will be assessed through the Perceived Stress Scale (Cohen et al., 1988). The Perceived Stress Scale (PSS) is the most widely used psychological instrument for measuring the perception of stress.

MOOD

The Brunel Mood Scale (BRUMS) is a 24-item self-report questionnaire developed by Terry, Lane, and Fogarty (2003), adapted from the Profile of Mood States (POMS) to assess six mood states—tension, depression, anger, vigour, fatigue, and confusion, each measured with four items. Respondents rate how they feel "right now" on a 5-point Likert scale from 0 (not at all) to 4 (extremely).

SLEEP QUALITY

Sleep Quality will be assessed through the Pittsburgh Sleep Quality Index. The Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989) is an effective instrument used to measure the quality and patterns of sleep-in adults.

TOTAL CHOLESTEROL, LDL, HDL, VLDL, TRIGLYCERIDES

To evaluate the selected lipid profile parameters, such as TC, LDL, HDL, VLDL, TG, the researcher will take the help of a lab assistant to collect samples from subjects, and the samples will be tested by a standardized laboratory method. The unit of measurement will be mg/dl.

ADMINISTRATION AND COLLECTION OF DATA

For the collection of data, Subjects will be Male university athletes , selected purposely. They will be randomly divided into two groups, Experimental group and Control group, consent form will be taken from subjects, all necessary information regarding the study

will be passed on ,those not interested or leaves the intervention in-between will be excluded then Psychological questionnaire of anxiety, stress, mood and sleep quality will be filled by subjects at the pre-test following all protocols, blood test will be taken in morning keeping empty stomach for 8 hour to check (TC, HDL, LDL, VLDL, Triglyceride) after that 6 week probiotic Supplementation will be given daily to the experimental group, at the end post data will be collected by psychological questionnaire of anxiety, stress, mood and sleep, blood test for selected lipid profile parameters will be collected to check changes after intervention.

STATISTICAL PROCEDURE

For the normality of the data, the Shapiro–Wilk and Kolmogorov-Smirnov tests will be applied. The mean and standard deviation will be used as descriptive statistics, and to compare the mean value between pre- and post-test of control and experimental group t-test will be applied. The level of significance will be set at 0.05.

EXPECTED OUTCOMES

The study is expected to show that probiotic supplementation significantly improves psychological variables (anxiety, stress, mood, and sleep quality) and physiological markers (lipid profile) in university athletes. It will provide scientific evidence supporting probiotics as a beneficial supplement for enhancing athletic performance and well-being, contributing valuable insights to sports science and health nutrition.

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